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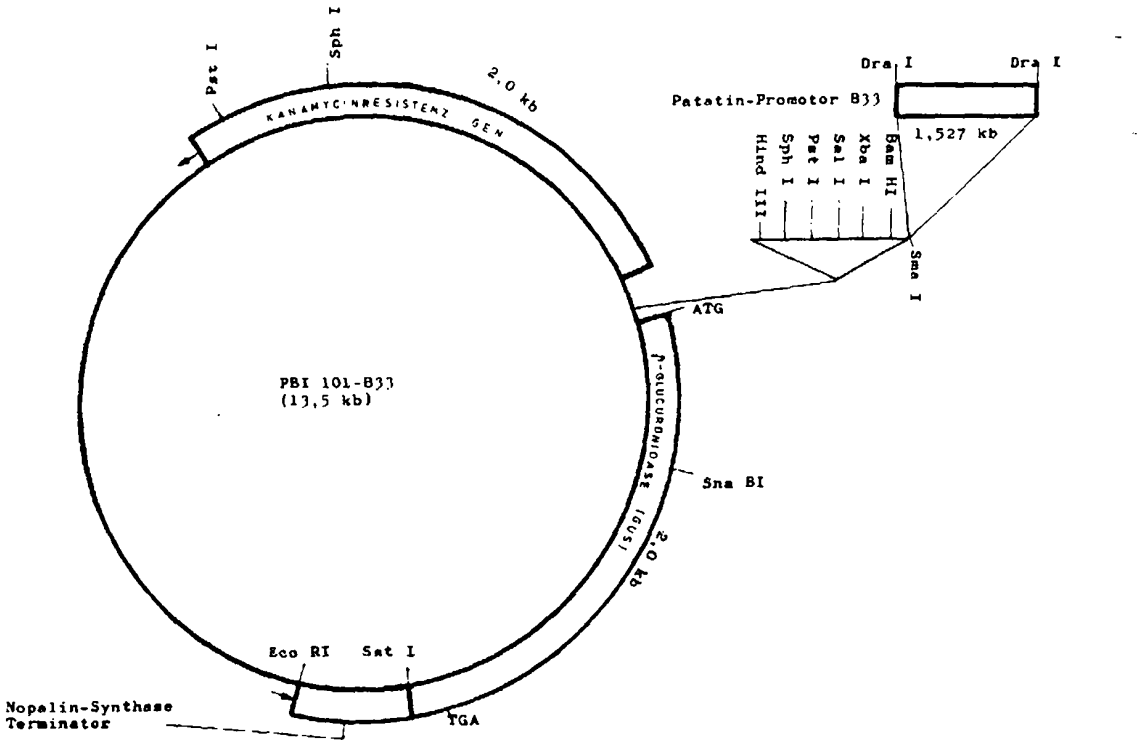
(54) **REGULATION TRANSCRIPTIONNELLE SPECIFIQUE DES TUBERCULES DE POMMES DE TERRE**

(54) **POTATOE TUBER SPECIFIC TRANSCRIPTIONAL REGULATION**



(57) There is described a new DNA sequence of an expression cassette on which the potato tuber specific regulatory regions are localised as well as the transfer of this DNA sequence into the plant genome using agrobacteria as transfer micro-organisms. The DNA sequence contains a patatin gene with a patatin gene promoter. The transfer DNA sequence acts both for regulating endogenous as well as for preparation of heterologous products in crops.

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ABSTRACT

There is described a new DNA sequence of an expression cassette on which the potato tuber specific regulatory regions are localised as well as the transfer of this DNA sequence into the plant genome using agrobacteria as transfer micro-organisms. The DNA sequence contains a patatin gene with a patatin gene promoter. The transfer DNA sequence acts both for regulating endogenous as well as for preparation of heterologous products in crops.

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This invention relates to a new DNA sequence of an expression cassette on which the potato tuber specific regulatory regions are localised as well as the transfer of this DNA sequence into the plant genome using agrobacteria as transfer micro-organisms. The DNA sequence contains a patatin gene with a patatin gene promoter. The transfer DNA sequence acts both for regulating endogenous as well as for preparation of heterologous products in crops.

Because of the continual increasing need for food and raw materials due to the growth in world population, and because of the long-term reduction in areas of land suitable for growing crops, it is becoming increasingly the task for biological research to to increase the yields of crops and their food content. An increase of yields can be achieved amongst other methods by increasing the resistance of crops against plant pests and plant diseases and/or poor soils. An increase of the resistance could achieved for example in such a way in that the plants induce and give rise to an increased formation of protective substances. For this, the metabolism of the plants must be manipulated. This can be achieved amongst other ways by changing the DNA contained in the cell nuclei. It would be desirable to act on in those DNA areas which are responsible for transcription in one or more of the parts of the plant or during a specified period in the plant growth cycle. For this there is a great interest in identifying the DNA sequence in the plant genome responsible for the transcription or expression of endogenous plant products. In order to find such DNA sequences, products first have to be sought which appear at a specific time in the cell growth cycle or in a

specific part of the plant. If the gene belonging to this is to be identified and isolated, a careful investigation of the sequence, and above all the identification and isolation of the desired transcriptional regulatory regions, is necessary. Suitable models must then be provided whose functions must established through experiments. Identifying such DNA sequences is a challenging project which is subject to substantial pitfalls and uncertainty. There is however substantial interest in the possibility of genetically modifying plants, which justifies the substantial expenditure and efforts necessary in identifying transcriptional sequences and manipulating them to determine their utility. Processes for genetic modification of dicotyledonous and monocotyledonous plants are known (EP 267159), as well as the following publications of Crouch et al., in: Molecular Form and Function of the Plant Genome, eds. van Vloten-Doting, Groots and Hall, Plenum Publishing Corp, 1985, pp 555-566; Crouch and Sussex, Planta (1981) 153:64-741 Crouch et al., J. Mol. Appl. Genet (1983) 2:273-283; and Simon et al., Plant Molecular Biology (1985) 5: 191-201, in which various forms of storage proteins in Brassica napus are described and by Beachy et al., EMBO. J. (1985) 4:3047-3053; Sengupta-Gopalan et al., Proc. Natl. Acad. Sci. USA (1985) 82:3320-3324; Greenwood and Chrispeels, Plant Physiol. (1985) 79:65-71 and Chen et al., Proc. Natl. Acad. Sci. USA (1986) 83:8560-8564, in which studies concerned with seed storage proteins and genetic manipulation are described and by Eckes et al., Mol. Gen. Genet. (1986) 205:14 - 22 and Fluhr et al., Science (1986) 232:1106-1112, in which genetic manipulation of light inducible plant genes are described.

There is now provided a DNA sequence of an expression cassette in which the potato tuber specific regulatory

regions are localised and which contain a patatin-gene with a patatin-gene promoter.

The DNA sequence, that contains the regulatory transcriptional starter region for the tuber specificity, can turn on a sequence, that contains the information for the modification of the phenotype of the third cell tissue and the formation both of quantitative distribution of endogenous products or the formation of heterogenous expression products for a new function. Conveniently, the transcription and termination regions in the direction of transcription should be provided by a linker or polylinker which contains one or more restriction positions for the insertion of this sequence. As a rule, the linker has 1-10, usually 1-8, preferably 2-6 reaction positions. In general the linker has a size of less than 100 bp, usually less than 60 bp, but is however at least 5 bp. The transcriptional starter region can be native or homologous to the host or foreign or heterologous to the host plants. Of special interest are the transcriptional starter regions which are associated with potatoes (Solanum tuberosum) proteinase-inhibitor II-gene, that during the total potato tuber development from the formation of the stolon up to the ripe tuber, is expressed. The transcription cassette contains in the 5'-3' transcription direction, a region representative for the plants for the transcription and the translation, a desired sequence and a region for the transcriptional and translational termination. The termination region is optionally exchangeable.

The DNA sequence could contain all possible open reading frames for a desired peptide as well as also one or more introns. Examples include sequences for enzymes; sequences that are complementary (a) to a genome sequence whereby

the genome sequence can be an open reading frame; (b) to an intron; (c) to a non-coded leading sequence; (d) to each sequence, which inhibits through complementarity, the transcription mRNA processing (for example splicing) or the translation. The desired DNA sequence can be synthetically produced or extracted naturally, or can contain a mixture of synthetic or natural DNA content. In general, a synthetic DNA sequence with codons is produced, which is preferred by the plants. This preferred codon from the plants can be specified from the codons with the highest protein frequency which can be expressed in the most interesting plant species. In the preparation of the transcription cassettes, the different DNA fragments can be manipulated in order to contain a DNA sequence, which leads generally in the correct direction and which is equipped with the correct reading frame. For the connections of the DNA fragments to each other, adaptors or linkers can be introduced on the fragment ends. Further manipulations can be introduced which provide the suitable restriction positions or separate the excess DNA or restriction positions. Where insertions, deletions or substitutions, such as for example transitions and transversions, are concerned, in vitro mutaganese, primer repair, restriction or ligation can be used.

In suitable manipulations, such as for example restriction, "chewing-back" or filling up of overhangs for "blunt-ends", complementary ends of the fragments for the fusing and ligation could be used. For carrying out the various steps which serve to ensure the expected success of the intervention, a cloning is necessary for the increase of the DNA amounts and for the DNA analysis.

A large amount of cloning vectors are available which contain a replication system in E. coli and a marker which

allows a selection of the transformed cells. The vectors contain for example pBR 332, pUC series, M13 mp series, pACYC 184 etc. In such a way, the sequence can be introduced into a suitable restriction position in the vector. The contained plasmid is used for the transformation in E. coli. The E. coli cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used a sequence analysis, a restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each plasmid sequence can be cloned in the same or different plasmid. After each introduction method of the desired gene in the plants further DNA sequences may be necessary. If for example for the transformation, the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced gene, can be connected. The use of T-DNA for the transformation of plant cells is being intensively studied and is well described in EP 120 516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, at al., Crit. Rev. Plant Sci., 4:1-46 und An et al., EMBO J. (1985) 4:277-284.

When the introduced DNA is first integrated once in the genome, it is then also relatively stable and as a rule no more comes out. It normally contains a selection marker which passes on to the transformed plant cells, resistance against a biocide or an antibiotic such as kanamycin, G 418, bleomycin, hygromycin or chloramphenicol, amongst others. The particular marker employed should be one which

will allow for selection of transformed cells compared to cells lacking the DNA which has been introduced.

A variety of techniques are available for introduction of DNA into a plant host cell. These techniques include transformation with T-DNA using Agrobacterium tumefaciens or Agrobacterium rhizogenes as transformation agent, the fusion, the injection or the electroporation as well as further possibilities. If Agrobacteria are used for the transformation, the introduced DNA must be cloned in special plasmid and either in an intermediary vector or a binary vector. The intermediary vectors which are based on sequences which are homologous with sequences in the T-DNA can be integrated through homologous re-combination in the Ti- or Ri- plasmid. These contain also the necessary Vir-region for the transfer of the T-DNA. Intermediary vectors cannot be replicated in Agrobacteria. By means of helper-plasmid, the intermediary vector of Agrobacterium tumefaciens can be transferred (conjugation). Binary vectors can be replicated in E. coli as well as in Agrobacteria. They contain a selection marker gene and a linker or polylinker, which are framed from the right and left T-DNA border regions. They can be transformed directly in the agrobacteria (Holsters et al., Mol. Gen. Genet.(1978) 163: 181-187). The Agrobacterium serving as host cells should contain a plasmid that carries the Vir-region, which is necessary for the transfer of the T-DNA in the plant cells whereby additional T-DNA can be contained. The bacterium so transformed is used for the transformation of plant cells. For the transfer of DNA in the plant cells, plant explanates can be cultivated in suitable manner with Agrobacterium tumefaciens or Agrobacterium rhizogenes. From the infected plant material (for example leaf bits, stem segments, roots as well as protoplasts or suspensions of cultivated cells), whole

plants can then be regenerated in a suitable medium which can contain antibiotics or biocides for the selection, which then can be tested for the presence of introduced DNA. In the injection and electroporation, no special requirements on the plasmid are needed and a simple plasmid, for example pUC derivative can be used.

For the introduction of foreign genes into plants there are many possibilities, but of especial interest is the expression of genes for mammalian products such as for example blood factors; lymphokines; colony stimulation factors; interferons; plasminogen activators, enzymes such as for example superoxide dismutase or chymosin; hormone; thioesterase-2 from rat milk or human serum albumin. A further possibility is increasing the amounts of tuber proteins, especially mutated tuber proteins, which show an optimised amino acid composition (essential amino acids) and in this way the nutritive value of the tubers can be increased. Should the amounts of specified endogenous products be reduced, the expression of the gene or parts of this gene in the wrong orientation to the promoter is also conceivable, which leads to synthesis of an RNA, which is complementary to a total or to parts of an endogenous gene and thus the transcription of this gene or the processing and/or translation of the endogenous mRNA can be inhibited.

The transformed cells grow within the plants in the usual way (see also McCormick et al., Plant Cell Reports (1986) 5, 81-84). These plants can be grown normally and crossed with plants, that possess the same transformed gene or other genes. The resulting hybridised individuals have the corresponding phenotypic properties. Two or more generations should be grown, in order to secure that the phenotypic state remains stable and will be passed on,

especially if seeds are to be harvested, in order to ensure that the corresponding phenotype or other individual characteristics are included. As host plants for the potato specific expression are all species or tuber forming plant species especially Solanum tuberosum.

The identification of necessary transcriptional starting regions can be achieved in a number of ways. There can be used as a rule mRNAs that are isolated from specific parts of plants (tubers). For the additional increase in concentration of the mRNA specific to the cells or associated with plant conditions, cDNA can be prepared whereby non-specific cDNA from the mRNA or the cDNA from other tissues or plant conditions (for example wounded/non-wounded) can be drawn off. The remaining cDNA can then be used for probing the genome for complementary sequences using a suitable plant DNA library. Where the protein is to be isolated, it can be partially sequenced so that a probe for direct identification of the corresponding sequences in a plant DNA library can be produced. The sequences that are hybridised with the probe can then be isolated and manipulated. Further, the non-translated 5'-region, that is associated with the coded area, can be isolated and used in expression cassettes for the identification of the transcriptional activity of the non-translated 5'-regions.

The expression cassette obtained, which the non-translated 5'-region uses, can be transformed in plants (see above) in order to test their functionability with a heterologous structure (other than the open reading frame of wild types which is associated with the non-translated 5'-region) as well as the tuber specificity. In this way can specific sequences that are not necessary for the tuber specific transcription, be identified. Expression cassettes that

are of especial interest contain transcriptional initiation positions of the patatin gene.

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Expressions & Abbreviations

Abbreviations:

d, kd = Dalton, kilodalton
bp = Base pairs
cDNA = A copy of a mRNA produced by reverse transcriptase.
mRNA = Messenger ribonucleic acid.
T-DNA = Transfer-DNA (localised on the Ti-plasmid from Agrobacterium tumefaciens)

Terms:

Blunt ends = DNA ends in which both DNA strands are exactly the same length.

Chewing-back = Enzymatic removal of nucleotides of a DNA strand which is longer than the complementary strand of a DNA molecule.

Electrophoresis = A biochemical process of separation for separating nucleic acids from proteins according to size and charge.

Expression = Activity of a gene.

Gene = Genetic factor; a unit of inheritance, carrier of part information for a particular specified characteristic. Genes consist of nucleic acids (eg DNA, RNA).

Genome = Totality of the gene localised in the chromosomes of the cell.

Genome-sequence = The DNA sequence of the genome whereby three nucleotide bases lying within it form a codon which code again for a specific amino acid.

RNA splicing = A gene does not always show up as a colinear unity but can contain non-coded sequences (introns) which must be spliced from the mRNA (splicing).

Heterologous gene(s) or DNA = Foreign genes or foreign DNA.

Homologous gene(s) or DNA = Gene or DNA derived from the same species.

Clone = Cell population that is derived from one of its own mother cells. Descendants are genotypically the same. By cloning, the homogeneity of cell lines can be increased further.

Ligation = Enzymatic formation of a phosphodiester bond between 5'-phosphate groups and 3'-hydroxy groups of the DNA.

Linker, Polylinker = Synthetic DNA sequence that contains one or more (polylinker) restriction cutting regions in direct sequence.

Northern blots, Southern blots, = Transfer and fixing of electrophoretically separate RNA or DNA on a nitrocellulose or nylon membrane.

Patatin = Trivial name for main storage protein of potato tubers; a glycoprotein of ca. kd molecular weight.

Phenotype = A sum of characteristics which expressed in an organism as opposed to its genotype.

Plasmid = Additional extrachromosomal DNA gene carrier in bacteria cells (possibly also in eukaryons) which reduplicate themselves independently of the

bacterial chromosomes. The plasmid can be integrated in other DNA hosts.

Primer = Starting piece; polynucleotide strand on which further nucleotides can be attached.

Promoter = Control sequence of the DNA expression which realises the transcription of homologous or heterologous DNA gene sequences.

Replication = Doubling of the DNA sequence.

Restriction enzymes = Restriction endonucleases that are in sub-units of the endo DNA's (for example EcoRI (specificity G↓AATTC and EcoRII↓CC ($^{A}_{T}$) GG, from E.coli) show themselves through a high specificity of the substrate knowledge (↓ = splitting position).

Restriction positions = A splitting position which is produced specifically by restriction enzymes.

Termination = A last stage of the protein and/or the RNA synthesis.

Transformation = Introduction of exogenous DNA of a bacterial species which is in a receiver cell.

Transcription = Overwriting on an RNA the genetic information contained in the DNA.

Translation = Translation of the genetic information which is memorised in the form of a linear sequence of bases in nucleic acids. The product of the translation is a polypeptide that comprises a

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sequence of amino acids.

Transition = Base pair exchange: purine-pyrimidine to purine-pyrimidine e.g. A-T exchanging G-C.

Transversion = Base pair exchange: purine-pyrimidine to pyrimidine-purine e.g. A-T replacing T-A.

Deletion = Removal of one or more base pairs;

Insertion = Introduction of one or more base pairs;

Transition, Transversion, Deletion and Insertion are point mutations.

Vectors = Host specific replicatable structures, that take up genes and carry these into other cells. Plasmid can also be used as vectors.

On 16.12.1988 the following microorganism was deposited at the German Collection for Microorganisms (DSM) in Braunschweig, Germany (deposit number):

Agrobacterium tumefaciens LBA4404, A. tum. M 14, containing the plasmid pBI 101-B33 (DSM 5089)

Description of the Figures

Figure 1 shows the restriction map of the genomic clone that codes the potato gene B33

Abbreviations:
E = Eco RI, H = HindIII, K = KpnI, B = Bam HI, S = SstI, V = Eco RV, X = XbaI, C = ClaI, D = DraI

Figure 2 shows the nucleic acid sequence for the transcriptional regulation of important areas of the patatin-gene.
In the sequence, the position of the DraI/DraI fragments between position +14 and position -1513, eg by Pfeil, is marked. ATG indicates the start of the translation (shown by ▼).

Figure 3 shows the 13.5 kb long plasmid PBI101-B33, with the 2.0.kb long kanamycin resistance gene, the 1.527 kb long patatin-promoter B 33, the 2.0.kb long β-glucuronidase resistance gene and the nopaline synthase terminator, contained within it.

For a better understanding of this invention the following examples are given. An explanation for these experiments is given as follows:

1. Cloning Vectors

For cloning, the vectors pUC18/19 (Yanisch-Perron et al Gene (1985), 33, 103-119) were used.

For plant transformations, the gene structures were cloned in the binary vector BIN19 (Bevan, Nucl Acids Research (1984), 12, 8711-8720).

2. Bacterial Species

For the pUC-and M13 vectors the E. coli species BMH71-18 (Messing et al, Proc. Nat. Acad. Sci. USA (1977), 24, 6342-6346) or TB1 was used. For the vectors pMPK110 and BIN19, the species TB1 was exclusively used. TB1 is a recombinant, negative, tetracyclines resistant derivative of the species JM101 (Yanisch-Perron et al., Gene (1985), 33, 103-119). The genotype of the TB1 species is (Bart Barrel, personal communication): F'(traD36, proAB, lacI, lacZΔM15), Δ(lac, pro), SupE, thiS, recA, Sr1::Tn10(Tc^R).

The plant transformation was carried out with the help of the Agrobacterium tumefaciens species LBA4404 (Bevan, M., Nucl. Acids Res. 12, 8711-8721 (1984); Bin19-derivative).

Medium

YT-Medium:	0.5% Yeast extract, 0.5% NaCl;. 0.8% bacto-trypton, if necessary in 1.5% agar.
YEB-Medium:	0.5% beef extract, 0.1% yeast extract, 0.5% peptone, 0.5% saccharose, 2 mM $MgSO_4$, if necessary in 1.5% agar.
MS-Medium:	According to Murashige and Skoog (Physiologia Plantarum (1962), 15, 473-497).

3. Transformation of agrobacterium tumefaciens.

The introduction of the DNA in the Agrobacterium in bin19-derivatives is carried out by direct transformation by the method of Holsters et a (Mol. Gen. Genet. (1978), 163, 181-187). The plasmid DNA transformed agrobacteria are isolated by the method of Birnboim and Doly (Nucl. Acids Res. (1979), 7, 1513-1523) and gel electrophoretically separated after suitable restriction cleavage.

4. Plant Transformation

10 small leaves of a sterile potato culture, wounded with a scalpel, were put into 10 ml MS-medium with 2% saccharose which contained 30 to 50 µl of an overnight culture of Agrobacterium tumefaciens, washed under selection. After 3-5 minutes gentle shaking, the petri dishes were incubated at 25°C in the dark. After two days, the leaves were laid in MS-medium with 1.6% glucose, 2 mg/l zeatinribose, 0.02 mg/l naphthylacetic acid, 0.02 mg/l gibberellic acid, 500 mg/l claforan, 50 mg/l kanamycin and 0.8% bacto-

agar. After one week incubation at 25°C and 3000 lux the claforan concentration in the medium was reduced by half.

5. Analysis of the Genomic DNA from Transgenic Plants

The isolation of genomic plant DNA was carried out by the method of Rogers and Bendich (Plant Mol. Biol (1985), 5, 69-76).

For DNA analysis 10-20 µg DNA was tested after suitable restriction cleavage with the aid of southern blots by integration of the DNA sequences being analysed.

6. Analysis of the Total RNA from Transgenic Plants

The isolation of the total plant RNA was carried out by the method of Longemann et al (Analytical Biochem (1987), 163, 16-2-).

For the analysis, 50 µg samples of total RNA were tested with the use of northern blots to determine the presence of the sought transcripts.

7. GUS-Test

The activity of the β-glucuronidase (GUS) in transgenic plants was determined by the method of Jefferson (Plant Mol. Biol. Rep. (1987), 5, 387-405). The protein determination was carried out by the method of Bradford (Anal. Biochem. (1976), 72, 248-254). For th determination of the gas activity, 50 µg Protein was used, and incubation was carried out at 37°C for 30 minutes.

The following examples illustrate the isolation and identification as well as the function and use of patatin promoters in potato tubers.

Example 1

Cloning and structural analysis of a patatin gene from Solanum tuberosum.

cDNA clones that code for the patatin protein in potatoes, were isolated and sequenced from the potato variety Berolina (Rosahl et al Mol. Gen. Genetics 203, 214-220 (1986). These cDNA clones then served to isolate a homologous genomic patatin clone from the potato variety Berolina (Max-Planck-Instut für Zuchtungsforschung, Köln).

Example 2

Cloning, identification and primary structure of a genomic patatin clone.

A genomic library of the nuclear DNA from the potato variety Berolina which was established in the vector from lambda phages EMBL 4, was screened using the patatin cDNA pcT 58. Thirteen independent clones were obtained which were used for the further work after partial sequencing of the clone B33. The restriction map of the clone B33 is shown in figure 1. Part of the gene was sequenced, the sequence of the important areas for the transcriptional regulation is given in figure 2.

Example 3

Identification of the regulatory regions responsible for the specific expression of the patatin gene B33.

A 1.527 kb long DraI/DraI fragment which is located between position +14 and position -1513 (see figure 2) was inserted in the SmaI cutting position of the plasmid pBO101 (Jefferson et al, EMBO J. 6, 3901-3907 (1987). In this was these promoter fragments of the patatin gene B53 with the coded region of the β-glucuronidase from E. coli and the poly-A containing region of the nopaline synthase gene were fused (see figure 3). These construction were transferred into the Agrobacterium species LBA 4404 (Bevan, M., Nucl. Acids Res. 12, 8711-8721 (1984) and the agrobacteria containing the chimeric patatin gene was used for transformation of potato leaves.

From ten independent containing transformants, in which the presence of the intact non-rearranged chimeric patatin glucuronidase gene was demonstrated, using southern blot analyses, leaves, stems, tubers and roots were analysed for activity of the β-glucuronidase.

The results are shown in Table 1. From these data it will be seen that the DraI/DraI fragment of the patatin gene B33 which was fused with the β-glucuronidase gene has a strong potato specific activity of the β-glucuronidase

Table 1

Glucuronidase of the chimeric B33 glucuronidase gene in various organs of different transgenic potato plants.

Transformant	Root	Stem	Leaf	Tuber
33G-13	137	55	0	16882
33G-19	138	7	14	2047
33G-21	155	1034	25	19471
33G-23	0	50	0	12149
33G-24	0	14	0	4530
33G-27	86	8	4	7284
33G-38	30	14	6	3847
33G-52	69	10	0	2864
33G-61	31	10	2	14916
33G-62	133	151	24	18620
x	76	135	7,5	11948

c.v. Desiree

Activities are given in pMol methylumbelliferrol/mg protein/minute

c.v. Desiree shows corresponding activity in an untransformed potato plant

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A process for the production of transgenic tuber forming plants, wherein said transgenic tuber forming plants express a DNA sequence of heterologous origin specifically in their tubers, said process comprising the following steps:

a) producing an expression cassette having the following sequences:

i) a B33 promoter sequence of a patatin gene derived from Solanum tuberosum, comprising the DraI/DraI fragment located between position +14 and position -1513 of the sequence of the KpnI/Hind III fragment shown in FIG. 2 and which leads to a tuber specific expression of sequences fused to said B33 promoter sequence,

ii) a DNA sequence of heterologous origin, which is fused in sense orientation to said B33 promoter sequence, and

iii) a DNA sequence for transcriptional and translational termination;

b) transferring said expression cassette into cells of said tuber forming plant, thereby producing transformed plant cells; and

c) regenerating whole, intact transgenic tuber forming plants from said transformed plant cells, wherein said transgenic tuber forming plants express the DNA sequence of heterologous origin specifically in their tubers.

2. A process according to claim 1, wherein said B33 promoter sequence comprises a DNA sequence consisting of:

1 TTTAAATCAT TGTTTTATTT TCTCTTTCTT TTTACAGGTA TAAAAGGTGA
51 AAATTGAAGC AAGATTGATT GCAAGCTATG TGTCACCACG TTATTGATAC
101 TTTGGAAGAA ATTTTTACTT ATATGTCTTT GTTTAGGAGT AATATTTGAT
151 ATGTTTTAGT TAGATTTTCT TGTCATTTAT GCTTTAGTAT AATTTTAGTT
201 ATTTTTATTA TATGATCATG GGTGAATTTT GATACAAATA TTTTTGTCAT
251 TAAATAAATT AATTTATCAC AACTTGATTA CTTTCAGTGA CAAAAAATGT
301 ATTGTCGTAG TACCCTTTTT TGTTGAATAT GAATAATTTT TTTTATTTTG
351 TGACAATTGT AATTGTCACT ACTTATGATA ATATTTAGTG ACATATATGT
401 CGTCGGTAAA AGCAAACACT TTCAGTGACA AAATAATAGA TTTAATCACA
451 AAATTATTAA CCTTTTTTAT AATAATAAAT TTATCCCTAA TTTATACATT
501 TAAGGACAAA GTATTTTTTT TATATATAAA AAATAGTCTT TAGTGACGAT
551 CGTAGTGTTG AGTCTAGAAA TCATAATGTT GAATCTAGAA AAATCTCATG
601 CAGTGTAAAA TAAACCTCAA AAAGGACGTT CAGTCCATAG AGGGGGTGTA
651 TGTGACACCC CAACCTCAGC AAAAGAAAAC CTCCCTTCAA CAAGGACATT
701 TGCGGTGCTA AACAATTTCA AGTCTCATCA CACATATATT TATTATATAA
751 TACTAATAAA GAATAGAAAA GGAAAGGTAA ACATCATTAA ATCGTCTTTG
801 TATATTTTTA GTGACAACTG ATTGACGAAA TCTTTTTCGT CACACAAAAT
851 TTTTAGTGAC GAAACATGAT TTATAGATGA TGAAATTATT TGTCCCTCAT
901 AATCTAATTT GTTGTAGTGA TCATTACTCC TTTGTTTGTT TTATTTGTCA
951 TGTTAGTCCA TTAAAAAAAA ATATCTCTCT TCTTATGTAC GTGAATGGTT
1001 GGAACGGATC TATTATATAA TACTAATAAA GAATAGAAAA AGGAAAGTGA
1051 GTGAGGTTCG AGGGAGAGAA TCTGTTTAAT ATCAGAGTCG ATCATGTGTC
1101 AATTTTATCG ATATGACCCT AACTTCAACT GAGTTTAACC AATTCCGATA
1151 AGGCGAGAAA TATCATAGTA TTGAGTCTAG AAAAATCTCA TGTAGTGTGG
1201 GGTAAACCTC AGCAAGGACG TTGAGTCCAT AGAGGGGGGT GTATGTGACA
1251 CCCCAACCTC AGCAAAAGAA AACCTCCCCT CAAGAAGGAC ATTTGCGGTG
1301 CTAAACAATT TCAAGTCTCA TCACACATAT ATATATATTA TATAATACTA
1351 ATAAATAATA GAAAAAGGAA AGGTAAACAT CACTAACGAC AGTTGCGGTG
1401 CAAACTGAGT GAGGTAATAA ACAGCACTAA CTTTTATTGG TTATGTCAAA
1451 CTCAAAGTAA AATTTCTCAA CTTGTTTACG TGCCTATATA TACCATGCTT
1501 GTTATATGCT CAAAGCACCA ACAAAATTT.

3. A process for the production of transgenic potato plants, wherein said transgenic potato plants express a DNA sequence of heterologous origin specifically in their tubers, comprising the following steps:

a) producing an expression cassette having the following sequences:

i) a B33 promoter sequence of a patatin gene derived from Solanum tuberosum, which is the DraI/DraI fragment located between position +14 and position -1513 of the sequence of the KpnI/Hind III fragment shown in FIG. 2 and which leads to a tuber specific expression of sequences fused to the B33 promoter sequence,

ii) a DNA sequence of heterologous origin, which is fused in sense orientation to said B33 promoter sequence, and

iii) a DNA sequence for transcriptional and translational termination;

b) transferring said expression cassette into potato cells thereby producing transformed potato cells; and

c) regenerating whole, intact transgenic potato plants from said transformed potato cells, wherein said transgenic potato plants express the DNA sequence of heterologous origin at a level at least 100 higher in their tubers than in their roots, stems, or leaves.

4. A plant cell containing an expression cassette having the following sequences:

i) a promoter sequence of a patatin gene derived from Solanum tuberosum comprising a DNA sequence consisting of:

1 TTTAAATCAT TGTTTTATTT TCTCTTTCTT TTTACAGGTA TAAAAGGTGA
51 AAATTGAAGC AAGATTGATT GCAAGCTATG TGTCACCACG TTATTGATAC
101 TTTGGAAGAA ATTTTTACTT ATATGTCTTT GTTTAGGAGT AATATTTGAT
151 ATGTTTTAGT TAGATTTTCT TGTCATTTAT GCTTTAGTAT AATTTTAGTT
201 ATTTTTATTA TATGATCATG GGTGAATTTT GATACAAATA TTTTTGTCAT
251 TAAATAAATT AATTTATCAC AACTTGATTA CTTTCAGTGA CAAAAAATGT
301 ATTGTCGTAG TACCCTTTTT TGTTGAATAT GAATAATTTT TTTTATTTTG
351 TGACAATTGT AATTGTCACT ACTTATGATA ATATTTAGTG ACATATATGT
401 CGTCGGTAAA AGCAAACACT TTCAGTGACA AAATAATAGA TTTAATCACA
451 AAATTATTAA CCTTTTTTAT AATAATAAAT TTATCCCTAA TTTATACATT
501 TAAGGACAAA GTATTTTTTT TATATATAAA AAATAGTCTT TAGTGACGAT
551 CGTAGTGTTG AGTCTAGAAA TCATAATGTT GAATCTAGAA AAATCTCATG
601 CAGTGTAAAA TAAACCTCAA AAAGGACGTT CAGTCCATAG AGGGGGTGTA
651 TGTGACACCC CAACCTCAGC AAAAGAAAAC CTCCCTTCAA CAAGGACATT
701 TGCGGTGCTA AACAATTTCA AGTCTCATCA CACATATATT TATTATATAA
751 TACTAATAAA GAATAGAAAA GGAAAGGTAA ACATCATTAA ATCGTCTTTG
801 TATATTTTTA GTGACAACTG ATTGACGAAA TCTTTTTCGT CACACAAAAT
851 TTTTAGTGAC GAAACATGAT TTATAGATGA TGAAATTATT TGTCCCTCAT
901 AATCTAATTT GTTGTAGTGA TCATTACTCC TTTGTTTGTT TTATTTGTCA
951 TGTTAGTCCA TTAAAAAAAA ATATCTCTCT TCTTATGTAC GTGAATGGTT
1001 GGAACGGATC TATTATATAA TACTAATAAA GAATAGAAAA AGGAAAGTGA
1051 GTGAGGTTCG AGGGAGAGAA TCTGTTTAAT ATCAGAGTCG ATCATGTGTC
1101 AATTTTATCG ATATGACCCT AACTTCAACT GAGTTTAACC AATTCCGATA
1151 AGGCGAGAAA TATCATAGTA TTGAGTCTAG AAAAATCTCA TGTAGTGTGG
1201 GGTAAACCTC AGCAAGGACG TTGAGTCCAT AGAGGGGGGT GTATGTGACA
1251 CCCCAACCTC AGCAAAAGAA AACCTCCCCT CAAGAAGGAC ATTTGCGGTG
1301 CTAAACAATT TCAAGTCTCA TCACACATAT ATATATATTA TATAATACTA
1351 ATAAATAATA GAAAAAGGAA AGGTAAACAT CACTAACGAC AGTTGCGGTG

1401 CAAACTGAGT GAGGTAATAA ACAGCACTAA CTTTTATTGG TTATGTCAAA
1451 CTCAAAGTAA AATTTCTCAA CTTGTTTACG TGCCTATATA TACCATGCTT
1501 GTTATATGCT CAAAGCACCA ACAAAATTT.

ii) a DNA sequence of heterologous origin, which is fused in sense orientation to said promoter sequence, and

iii) a DNA sequence for transcriptional and translational termination.

5. A plant cell according to claim 4, wherein the patatin promoter sequence has a DNA sequence consisting of:

1 TTTAAATCAT TGTTTTATTT TCTCTTTCTT TTTACAGGTA TAAAAGGTGA
51 AAATTGAAGC AAGATTGATT GCAAGCTATG TGTCACCACG TTATTGATAC
101 TTTGGAAGAA ATTTTTACTT ATATGTCTTT GTTTAGGAGT AATATTTGAT
151 ATGTTTTAGT TAGATTTTCT TGTCATTTAT GCTTTAGTAT AATTTTAGTT
201 ATTTTTATTA TATGATCATG GGTGAATTTT GATACAAATA TTTTTGTCAT
251 TAAATAAATT AATTTATCAC AACTTGATTA CTTTCAGTGA CAAAAAATGT
301 ATTGTCGTAG TACCCTTTTT TGTTGAATAT GAATAATTTT TTTTATTTTG
351 TGACAATTGT AATTGTCACT ACTTATGATA ATATTTAGTG ACATATATGT
401 CGTCGGTAAA AGCAAACACT TTCAGTGACA AAATAATAGA TTTAATCACA
451 AAATTATTAA CCTTTTTTAT AATAATAAAT TTATCCCTAA TTTATACATT
501 TAAGGACAAA GTATTTTTTT TATATATAAA AAATAGTCTT TAGTGACGAT
551 CGTAGTGTTG AGTCTAGAAA TCATAATGTT GAATCTAGAA AAATCTCATG
601 CAGTGTAAAA TAAACCTCAA AAAGGACGTT CAGTCCATAG AGGGGGTGTA
651 TGTGACACCC CAACCTCAGC AAAAGAAAAC CTCCCTTCAA CAAGGACATT
701 TGCGGTGCTA AACAATTTCA AGTCTCATCA CACATATATT TATTATATAA
751 TACTAATAAA GAATAGAAAA GGAAAGGTAA ACATCATTAA ATCGTCTTTG
801 TATATTTTTA GTGACAACTG ATTGACGAAA TCTTTTTCGT CACACAAAAT
851 TTTTAGTGAC GAAACATGAT TTATAGATGA TGAAATTATT TGTCCCTCAT
901 AATCTAATTT GTTGTAGTGA TCATTACTCC TTTGTTTGTT TTATTTGTCA
951 TGTTAGTCCA TTAAAAAAAA ATATCTCTCT TCTTATGTAC GTGAATGGTT
1001 GGAACGGATC TATTATATAA TACTAATAAA GAATAGAAAA AGGAAAGTGA
1051 GTGAGGTTCG AGGGAGAGAA TCTGTTTAAT ATCAGAGTCG ATCATGTGTC

1101 AATTTTATCG ATATGACCCT AACTTCAACT GAGTTTAACC AATTCCGATA
1151 AGGCGAGAAA TATCATAGTA TTGAGTCTAG AAAAATCTCA TGTAGTGTGG
1201 GGTAAACCTC AGCAAGGACG TTGAGTCCAT AGAGGGGGGT GTATGTGACA
1251 CCCCAACCTC AGCAAAAGAA AACCTCCCCT CAAGAAGGAC ATTTGCGGTG
1301 CTAAACAATT TCAAGTCTCA TCACACATAT ATATATATTA TATAATACTA
1351 ATAAATAATA GAAAAAGGAA AGGTAAACAT CACTAACGAC AGTTGCGGTG
1401 CAAACTGAGT GAGGTAATAA ACAGCACTAA CTTTTATTGG TTATGTCAAA
1451 CTCAAAGTAA AATTTCTCAA CTTGTTTACG TGCCTATATA TACCATGCTT
1501 GTTATATGCT CAAAGCACCA ACAAAATTT.

6. Agrobacterium tumefaciens LBA 4404 A-tum B33 (DSM 5089).

7. Use of a promoter sequence comprising a nucleotide sequence consisting of:

1 TTTAAATCAT TGTTTTATTT TCTCTTTCTT TTTACAGGTA TAAAAGGTGA
51 AAATTGAAGC AAGATTGATT GCAAGCTATG TGTCACCACG TTATTGATAC
101 TTTGGAAGAA ATTTTTACTT ATATGTCTTT GTTTAGGAGT AATATTTGAT
151 ATGTTTTAGT TAGATTTTCT TGTCATTTAT GCTTTAGTAT AATTTTAGTT
201 ATTTTTATTA TATGATCATG GGTGAATTTT GATACAAATA TTTTTGTCAT
251 TAAATAAATT AATTTATCAC AACTTGATTA CTTTCAGTGA CAAAAAATGT
301 ATTGTCGTAG TACCCTTTTT TGTTGAATAT GAATAATTTT TTTTATTTTG
351 TGACAATTGT AATTGTCACT ACTTATGATA ATATTTAGTG ACATATATGT
401 CGTCGGTAAA AGCAAACACT TTCAGTGACA AAATAATAGA TTTAATCACA
451 AAATTATTAA CCTTTTTTAT AATAATAAAT TTATCCCTAA TTTATACATT
501 TAAGGACAAA GTATTTTTTT TATATATAAA AAATAGTCTT TAGTGACGAT
551 CGTAGTGTTG AGTCTAGAAA TCATAATGTT GAATCTAGAA AAATCTCATG
601 CAGTGTAAAA TAAACCTCAA AAAGGACGTT CAGTCCATAG AGGGGGTGTA
651 TGTGACACCC CAACCTCAGC AAAAGAAAAC CTCCCTTCAA CAAGGACATT
701 TGCGGTGCTA AACAATTTCA AGTCTCATCA CACATATATT TATTATATAA
751 TACTAATAAA GAATAGAAAA GGAAAGGTAA ACATCATTAA ATCGTCTTTG
801 TATATTTTTA GTGACAACTG ATTGACGAAA TCTTTTTCGT CACACAAAAT
851 TTTTAGTGAC GAAACATGAT TTATAGATGA TGAAATTATT TGTCCCTCAT

901 AATCTAATTT GTTGTAGTGA TCATTACTCC TTTGTTTGTT TTATTTGTCA
951 TGTTAGTCCA TTAAAAAAAA ATATCTCTCT TCTTATGTAC GTGAATGGTT
1001 GGAACGGATC TATTATATAA TACTAATAAA GAATAGAAAA AGGAAAGTGA
1051 GTGAGGTTCG AGGGAGAGAA TCTGTTTAAT ATCAGAGTCG ATCATGTGTC
1101 AATTTTATCG ATATGACCCT AACTTCAACT GAGTTTAACC AATTCCGATA
1151 AGGCGAGAAA TATCATAGTA TTGAGTCTAG AAAAATCTCA TGTAGTGTGG
1201 GGTAAACCTC AGCAAGGACG TTGAGTCCAT AGAGGGGGGT GTATGTGACA
1251 CCCCAACCTC AGCAAAAGAA AACCTCCCCT CAAGAAGGAC ATTTGCGGTG
1301 CTAAACAATT TCAAGTCTCA TCACACATAT ATATATATTA TATAATACTA
1351 ATAAATAATA GAAAAAGGAA AGGTAAACAT CACTAACGAC AGTTGCGGTG
1401 CAAACTGAGT GAGGTAATAA ACAGCACTAA CTTTTATTGG TTATGTCAAA
1451 CTCAAAGTAA AATTTCTCAA CTTGTTTACG TGCCTATATA TACCATGCTT
1501 GTTATATGCT CAAAGCACCA ACAAAATTT.

for tuber specific expression in cultivated plants.

8. Use of the promoter sequence according to claim 7, for the regulation of expression of endogenous products or for the production of heterologous products in cultivated plants.

Fig. 1

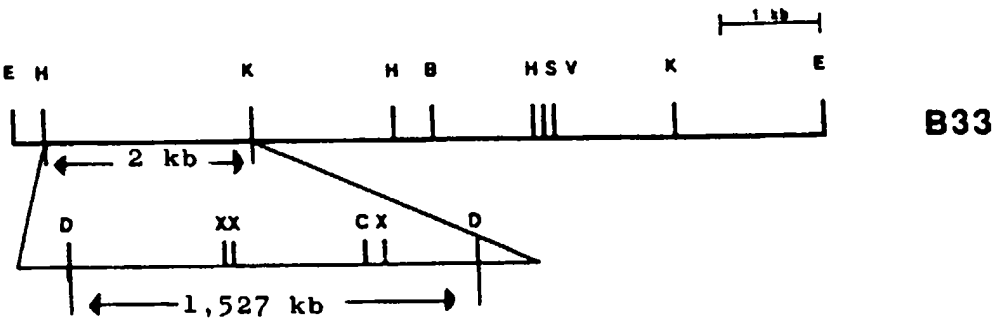

Fig. 2

CA2006454

Wagner & Black

CA2006454

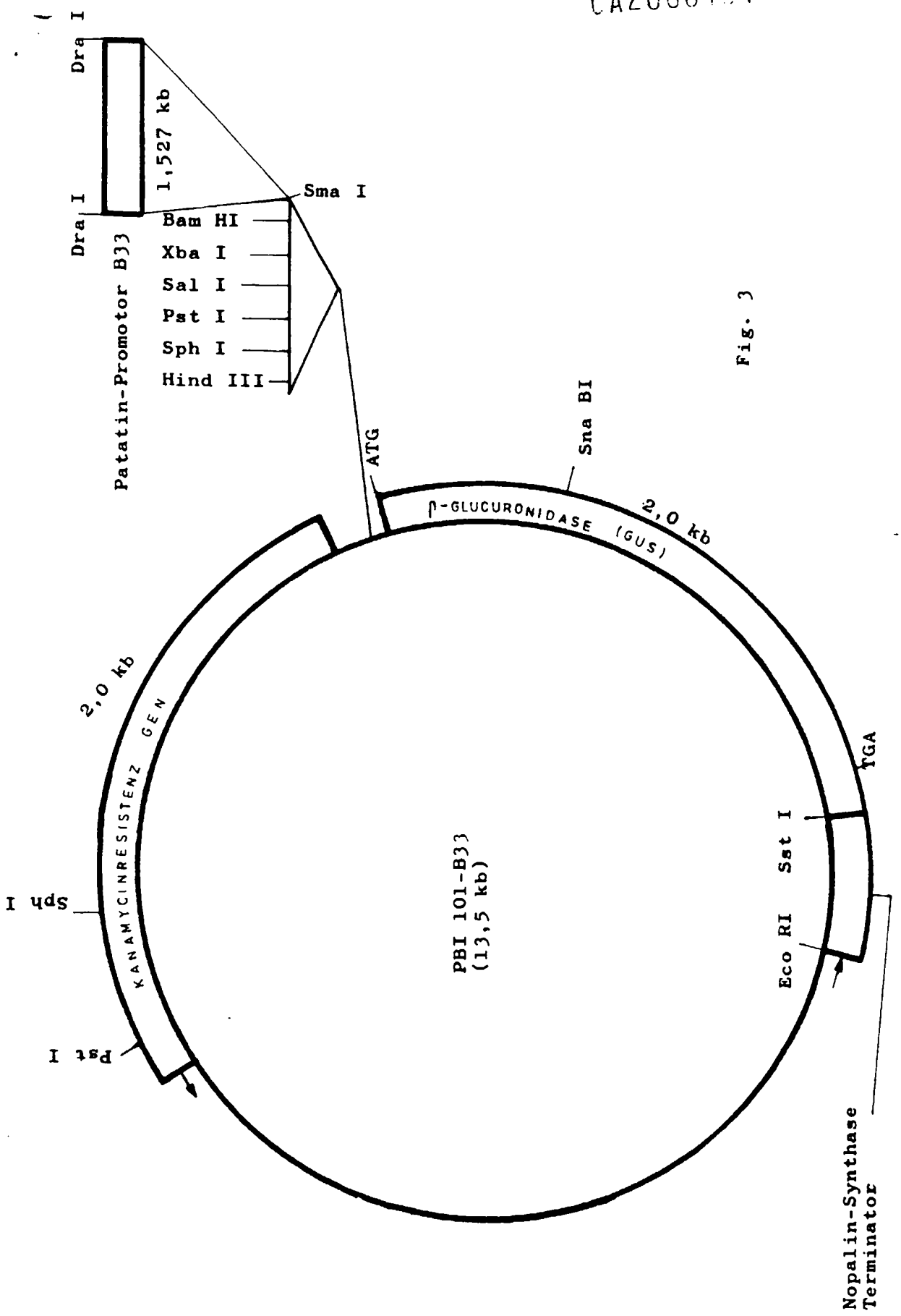

Fig. 3